RESEARCHES

(4)

ON

MYOHÆMATIN AND THE HISTOHÆMATINS.

BY

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VI. *Researches on Myohæmatin and the Histohæmatins.*

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[Plates 11, 12.]

In a short paper read before the Physiological Society* in 1884 I gave a preliminary account of a new colouring matter which I had discovered in muscle by means of the spectroscope; also of a class of colouring matters found in the tissues and organs of invertebrate and vertebrate animals to which the former pigment evidently belongs, and which I named histohæmatins from their occurrence in the animal tissues. The name myohæmatin was proposed for the muscle pigment for reasons which will be given further on.

Since the publication of that paper I have been engaged in working out the distribution of these pigments in the Animal Kingdom, and have tried to find out their relationship to other colouring matters and the changes produced in them by reagents.

Methods of Investigation.—These colouring matters have been missed by other observers owing to their not having adopted the methods of examination used by me. The portion of tissue or organ under consideration is examined in a "compressorium," by means of which any required thickness can be examined; it is illuminated by a strong light condensed upon it by means of a substage achromatic condenser, and is examined by a spectrum eye-piece in a binocular microscope. The advantage of using a binocular instrument consists in this:—That one of the tubes is used as a finder, and in enabling one to see what is furnishing the spectrum. A one-inch objective is the most suitable power for this purpose, though sometimes the $\frac{1}{8}$th or $\frac{1}{4}$th may be required. The objectives have been shortened and the condenser so arranged as to allow of the full illumination of both fields when the $\frac{1}{4}$th and $\frac{1}{8}$th objectives are used. A Swan lamp was sometimes used, sometimes direct sunlight, but in many cases a good Argand gas-burner is sufficient. Observations were frequently checked (and measurements made in wave-lengths) by bringing the object in the compressorium before the slit of a large one-prism chemical spectroscope,† and I have latterly

* Proc. Physiol. Soc., 1884, No. IV., December 13.

† Although all the following measurements were made with the same instrument, and are, therefore, valuable for comparison, *inter se*, yet, owing to the great difficulty of seeing these bands, they may require slight modifications by and by.

derived great assistance by the use of an instrument provided with shutters in the eye-piece by means of which any portion of the spectrum can be cut off, which was made by ADAM HILGER. When an object is examined in the chemical spectroscope it has to be illuminated by a bull's-eye condenser placed between it and the light-source.

Animals Examined.—My observations have been confined necessarily to such animals as can be procured in an inland place, but the number examined is sufficient to prove the wide distribution and importance of the pigments to be described. Had my opportunities been greater the results would doubtless have been more important.

Among invertebrates various mollusca, echinoderms, arthropods, and some worms have been examined ; the results of the examination of actiniæ have been already communicated to the Royal Society. Among vertebrates various reptiles, amphibians, fishes, birds, and mammals have been examined, and the results obtained show that there is no essential difference in the spectra of the organs and tissues of vertebrates and invertebrates, when the influence of the circulating hæmoglobin in the former has been eliminated.

I. THE HISTOHÆMATINS.

In Dr. SORBY'S paper on the "Evolution of Hæmoglobin"* a remark occurs which shows that he had seen a spectrum which is that of a histohæmatin. Speaking of the hæmatin first observed by him in the bile of *Helix* he says :—"I think it very probably does occur in the muscles of the foot and elsewhere," &c. Here at least a previous observer—whose opinion is of great weight owing to his skill in chromatology—suspected the existence of a hæmatin-like pigment in the tissues of an animal, and believed it to be related to the hæmatin of the bile of the same animal. Not only, however, does a histohæmatin occur in an animal whose "bile" shows a hæmochromogen-like spectrum, but also in other animals in which no hæmochromogen can be detected in the "bile," and the bands of the respective histohæmatins are absolutely identical. Moreover we find the histohæmatin spectrum replaced by that of a hæmochromogen-like spectrum in the solid organs and tissues in individual cases; it is for that reason that I have named these pigments histohæmatins. No other observers, so far as I know—except Dr. SORBY and myself—have seen, or at least have recorded, the occurrence of such a spectrum in the organs or tissues of an animal.

Histohæmatins of Echinodermata.

The tissues and organs of various echinoderms have been examined, and in most of them the appearances differ in no respect from those seen in *Uraster rubens.* In this species the spectra are well marked, and may be taken as typical, their bands being very sharp and easily seen.

If we compare the spectrum of the generative organs of *Uraster* with that of

* Quart. Journ. Micros. Soc., vol. XVI., New Series, pp. 77, *et seq.*

muscle, or with that of the blood-free pancreas of a cat, a most striking resemblance is apparent. Such a statement may appear startling but is nevertheless true, as I have convinced myself by repeated examination and measurements. The eggs of most animals when examined by this method show the lutein band or bands, but in *Uraster* the spectrum is entirely different and belongs to a typical histohæmatin.* We find a narrow band before D, two still narrower between D and E, and two others faint nearer the violet, spectrum 1, Chart I. They gave the following readings (approximately):—

1st band . . .	λ 613	to	λ 591, or 593,
2nd band . . .	λ 569	,,	λ 560,
3rd band . . .	λ 556	,,	λ 548·5,
4th band . . .	λ 537	,,	λ 516 (about).

A great number of specimens were examined and in all the same spectrum observed.

The stomach-wall likewise gave a distinct spectrum which bears a close resemblance to hæmochromogen, spectrum 2, Chart I. The first of these bands read λ 566 to λ 558·5 (approximate). There is evidence to show that elsewhere in this species a histohæmatin occurs ; thus the spectrum of the ampullæ resembles that of the stomach-wall.

I tried by various solvents to isolate these pigments but failed. The colour of the organs in which they occur is a more or less pale-yellow. The bands appear to become darker on treatment with reducing agents, and less shaded on exposure to the air, although the change from the oxidised to the reduced state is not easily brought about.

In the integument of *Uraster rubens*, when it has a brownish tint, the presence of hæmatoporphyrin† can be easily proved, and as the only pigments present in the animal are enterochlorophyll in the radial cœca, histohæmatins in the tissues, and a lipochrome here and there, and as hæmatoporphyrin cannot be obtained from enterochlorophyll or from the lipochromes, it is highly probable it is a metabolite of the histohæmatins; or what is less likely that these pigments may be derived from the same radical.

In *Ophiactis virens*, M. FŒTTINGER‡ states that he found hæmoglobin, but KRUKENBERG § thinks this observation incorrect, and that the water-vascular system of this echinoderm probably contains a pigment related to the bile-hæmatin ("helico-rubin"

* A small quantity of a lutein (=lipochrome) can be got out of the eggs by absolute alcohol. The male generative organs contain histohæmatin as well as the eggs.

† It occurs in the integument in the condition of hæmatoporphyrin as such, as can easily be proved by examining the scraped-off pigment with the microspectroscope. I first showed its presence in this situation. Proc. Birm. Phil. Soc., vol. iii., p. 378. It can be isolated by digesting the integument in alcohol and sulphuric acid. Journ. Physiol. vol. vii., pp. 240–252.

‡ Bull. Belgian Acad., No. 5.

§ "Grundzüge einer vergleichenden Physiologie der Farbstoffe," &c., p. 134.

of KRUKENBERG, my enterohæmatin) of *Helix*. I was inclined at first to agree with KRUKENBERG for the reasons given above.*

Histohæmatins of Mollusca.

In this sub-kingdom one meets with appearances which prove the great importance of the histohæmatins from a physiological point of view, and prove their connexion with, and replacement by, pigments which are known to be connected with hæmoglobin and its derivatives. SORBY'S remarks on the presence of certain bands in the foot of *Helix* have been given above.

Littorina littorea.—Hæmoglobin occurs in the pharyngeal muscle,† and I have changed it into hæmatin, both acid and alkaline, it here *occupies the place of myohæmatin* as a comparison with other species shows.

In the yellower parts of the foot two faint bands like those of hæmochromogen can be detected. In other fleshy parts of the body we get a spectrum which resembles myohæmatin, if we suppose the second and third band of its spectrum to have coalesced (spectrum 3, Chart I.).

Purpura lapillus.—Hæmoglobin occurs in the pharyngeal muscle replacing the myohæmatin of other species.

In the foot, a histohæmatin spectrum is visible, resembling that of *Littorina* (spectrum 4, Chart I.). In other parts of the animal no bands are with certainty visible, except in the liver (referred to in former papers), but here and there a faint histohæmatin spectrum can be just seen.

Trochus cinerarius.—The pharyngeal muscle contains hæmoglobin. Contrary to what I found in *Littorina*, it is absent from the radula. Faint traces of a histohæmatin are present, but none distinct enough to map.

Patella vulgata.—Hæmoglobin occurs in the pharyngeal muscle, and in the radula in traces. The foot shows a distinct histohæmatin spectrum, and in other parts faint traces of its bands are just visible.

In most specimens these bands resembled closely those of hæmochromogen, and, since the latter is present in its "bile," as I have shown, there can hardly be a doubt that this pigment is taken up from the intestine and deposited in the tissues as a histohæmatin.

In *Limnæus stagnalis* and *Paludina vivipara*, besides the hæmoglobin in the pharyngeal muscles, there is evidence elsewhere of the presence of a histohæmatin, but the examination of these species is yet incomplete.

In *Mytilus edulis*, in the mantle, a distinct histohæmatin spectrum is apparent, and on comparing this with the spectrum of myohæmatin and with other histohæmatins a very striking likeness is apparent.‡ In the ovaries a somewhat similar spectrum is

* HOWELL has, however, recently found hæmoglobin in a *Holothurian*, which tends to support FŒTTINGER'S statement. June 16, 1886.—JOHNS HOPKINS University Circular, v., 1885.

† And in the radula. In the liver of some I have detected bands like those of hæmochromogen.

‡ After STOKES'S fluid the bands read :—1st λ 613–596·5; 2nd, λ 569–563; 3rd, λ 556–550.

seen, differing from that of the mantle in this that the two second bands are fused into one. (See Chart I., spectra 5 and 6.) In the heart there is an attempt at a myohæmatin spectrum. In the gills, and in the adductor and retractor muscles, faint bands may be seen in some specimens belonging to the histohæmatins.

In *Ostræa edulis* the fleshy abdomen gives a distinct, though faint, histohæmatin spectrum :—a faint band before D, and another in green like the first band of hæmochromogen. On treatment with sulphide of ammonium the spectrum is better defined. On examining two or three thicknesses of mantle placed over each other a similar spectrum is seen. A similar pigment occurs in the gills, only that the band before D is invisible. In the stomach wall, intestine, and in the adductor muscle we meet with similar appearances. It requires a practised eye to recognise these bands with certainty.

In other lamellibranchiate mollusca, such as *Unio* and *Anodonta*, histohæmatins occur especially in the foot, but it is in the pulmonate mollusca that their spectra are most distinct. Just as in birds and in insects, where respiratory activity is greatest, we find the myohæmatin spectrum best marked, so in the air-breathing mollusca the histohæmatins are better marked than in other members of their sub-kingdom. It is worthy of notice that in *Helix*, *Limax*, *Arion*, and others the "bile" contains enterohæmatin, and that in *Limax* and *Arion*, just as in *Uraster rubens*, the integument can be made to yield hæmatoporphyrin as I have shown.* I think here, too, there can be little doubt that the histohæmatins are the mother substance of the integumental hæmatoporphyrin. In the heart of all the pulmonate mollusca and in their buccal muscles myohæmatin occurs, as will be shown further on.

In *Helix aspersa* (besides the heart and pharyngeal muscle) spectra are perceptible in the *dartsac*, in the *ovo-testis*, *vas deferens*, *albumen gland*, in the *foot*, in the wall of *crop*, in the *receptaculum seminis*, in the *oviduct*, in the *penis*, in the *nephridium*, and in the *finger-shaped gland* (CLAUS). In most of these a faint band before D occurs, and either one broad band occupying the position of the second and third myohæmatin bands, or one or two faint bands resembling and placed nearly in the position of those of hæmochromogen. The spectra correspond so closely with those seen in *Limax* that those figured for *Limax* will illustrate the above.

In the *receptaculum seminis* of one *Helix* a redder portion was visible, which showed spectrum 7, Chart I., and which is remarkable for its likeness to methæmoglobin. In the *nephridium* the spectrum was well marked, and either showed a feeble band before D and a broader and darker one between D and E, spectrum 8, Chart I. (from *Limax*), or two narrow bands in green closely resembling the second and third of myohæmatin (spectrum 9, Chart I.).

In *Helix pomatia* similar spectra can be observed. In the foot a band, or sometimes two, corresponding to those of hæmochromogen, are visible ; a similar spectrum in the *dartsac*, and similar spectra in the same parts as those in *Helix aspersa*. *Limax*

* Proc. Birm. Phil. Soc., *loc. cit.*, and Journ. of Physiology, vol. vi., p. 38; vol. vii., pp. 240–252.

shows similar spectra. In the heart and pharyngeal muscles myohæmatin occurs; in the *nephridium* spectrum 8, Chart I., is seen; in the *ovo-testis* a faint band before D and a narrow one in green like the first of hæmochromogen; in the albumen gland a similar spectrum (spectrum 10, Chart I.); and the *vas deferens* gave spectrum 11, Chart I., resembling that of the *foot* closely. All specimens of *Limax maximus L. flavus* and *L. variegatus* presented similar appearances.

In *Arion ater* the *nephridium* showed a spectrum resembling that of myohæmatin (*cf.* spectrum 9, Chart I.), while the spectra of its other organs are the same as those in *Limax*. The spectrum of the nephridium is remarkable for its resemblance to that of the kidney of vertebrates.

Histohæmatins of Arthropoda.

In this sub-kingdom the histohæmatins are the same as those already described; they are well marked in the crustacea, of which *Homarus vulgaris, Cancer pagurus, Carcinus mænas, Astacus fluviatilis* and *Pagurus bernhardus* have been examined. In the hearts of all these I have found myohæmatin, which will be referred to further on. Among *Arachnida* I have examined *Epeira diadema,** *Tegenaria civilis* and other spiders in which myohæmatin occurs, but owing to the smallness of their parts the occurrence of other histohæmatins although very probable is not quite certain. In *Insecta* we meet with *myohæmatin* in abundance, but owing to the thinness of the intestine and the small size of the *ovaries, malpighian tubes*, &c., one cannot be certain as to the presence of the histohæmatins. There are, however, exceptions to this statement, which will be referred to again.

Astacus fluviatilis.—In this species enterohæmatin is found in the bile, as already described by me,† myohæmatin in the heart, but apparently not in the voluntary muscles.

In the yellow *stomach-wall*, after washing well with water, the spectrum in some cases shows that figured in spectrum 12, Chart I. This is remarkably like that seen in the nephridium of *Arion* and in the kidney of higher animals (vertebrates). In other specimens this spectrum is replaced by another in which the narrow band in green is like that found in *Uraster*, but this, from what has been already shown, is merely a modification of the other (spectrum 13). In the green gland (nephridium) of all the specimens examined the same spectrum is seen, and is shown in Chart I., spectrum 14. The branchiæ sometimes show a hæmochromogen-like spectrum (spectrum 15, Chart I.). The ovaries only gave a lutein-like spectrum.

In *Homarus vulgaris* one finds myohæmatin present in the muscle of the heart, but apparently absent in the voluntary muscles. The *stomach-wall* shows a faint band before D, and another faint and narrow one in green similar to those seen in the

* The livers of these spiders show a distinct band before D and a darker one in the green, therefore a histohæmatin spectrum; *cf. Carcinus.*

† Proc. Roy. Soc., vol. 35, p. 381.

same situation in the crayfish. The *green-gland* gives the same spectrum as that of the latter species also (spectrum 16, Chart I.). In the *vas deferens* and the *branchiæ* no bands could be detected.

In *Cancer pagurus* and *Carcinus mœnas* similar spectra* were observed. In *Pagurus bernhardus*, whose heart muscle contains myohæmatin, there are faint traces of histohæmatins, but not distinct enough to map their spectra.

Among *Insecta*, as already stated, the presence of histohæmatins is difficult to determine. In the crop and other parts of the enteron faint bands coincident with those of histohæmatin may be seen in some species ; one might expect to meet with myohæmatin in this situation owing to the muscle of the intestine being striped in insects, but hitherto I have not found it. In the mouth parts of some larvæ myohæmatin occurs (*e.g.* Ennomos), and, as will be shown again, all adult insects possess myohæmatin in their voluntary muscles.

In *Staphylinus olens* we meet with a most remarkable spectrum, yielded by the testes, which shows that the hæmoglobin-forming tendency is not altogether suppressed in insects. I have not met with this appearance in other species.

In the specimens of *Staphylinus* examined the testes were a fine carmine colour ; squeezed out in the compressorium, and examined in the usual manner, they gave two bands possessing a remarkable resemblance to those of reduced hæmatin (spectrum 17, Chart I.). Before squeezing down the cover-glass of the compressorium the spectrum resembled oxyhæmoglobin, but in a suitable thickness the bands appeared as shown. It will be noticed the first band is placed over a shading which occupies nearly the position of the band of reduced hæmoglobin. The position of these bands is approximately : 1st, λ 569 to λ 554·5, and 2nd, λ 540 to λ 524·5. I succeeded in converting this pigment into acid hæmatin by the action of acetic acid, by which the bright red colour was changed to a dull brown and the spectrum into that of acid hæmatin. As spermatozoa were easily seen in these organs with the microscope there can be no doubt as to their nature.

Vermes.

In *Lumbricus*† and *Hirudo* all the organs which in other species show histohæmatin spectra appear to contain a small amount of hæmoglobin, which doubtless functions in a similar manner to the histohæmatins.

In *Aphrodite*, besides the hæmoglobin contained in the ventral chain of ganglia, I have detected traces in the muscular fore-gut.

Histohœmatins in Vertebrata.

In *Vertebrata* the search for histohæmatins in the organs and tissues is attended with difficulty, owing to the presence of hæmoglobin, but fortunately the bands of the

* The *liver* of *Carcinus mœnas* shows a band before D and a dark one in green.

† *Lumbricus* contains hæmatoporphyrin in the dorsal streak. For *Serpula* and *Sabella* see my paper "On the Chromatology of the Blood of some Invertebrates," Quart. Journ. Micros. Science., vol. C., p. 469.

histohæmatins can be recognised in the tissues or organs when squeezed out to a degree of thinness which no longer allows the bands of hæmoglobin to be seen, and in most cases the blood-vessels can be injected with salt solution sufficiently to eliminate the influence of the circulating hæmoglobin.

Although Birds and Mammals present the best marked histohæmatin spectra, yet to preserve the continuity of this paper *Fishes* will be dealt with first. I will, however, deal briefly with this class, as the spectra of their organs are not as well marked as in other classes. In the golden carp (*Cyprinus*), the mackerel (*Scomber scombrus*), the herring (*Clupea heringus*), the roach (*Leuciscus rutilus*), and the tench (*Tinca vulgaris*), the stomach-wall, liver, kidney, and intestinal wall, and perhaps other organs, present histohæmatin spectra. In some the spectra are obscured by the presence of oxyhæmoglobin, which appears sometimes to replace them.

The spectrum yielded by the liver and kidney is that shown in spectrum 1, Chart II.* In some parts of the kidney the band shown there is apparently replaced by two resembling in this point the spectrum of the kidney in other animals.

In other fishes similar spectra were observed, and in every species examined I found myohæmatin.

Amphibians.

In *Rana temporaria* a well-marked histohæmatin spectrum can be seen in the *stomach-wall*, spectrum 2, Chart II. The *testis* appears to contain traces of the same bands. The *liver* shows a band covering D, and a darker between D and E. In the *spleen*, besides that of reduced hæmoglobin a similar spectrum occurs, also in the *kidney*.

In *Bufo vulgaris* the same organs show similar spectra.

In *Hyla arborea* there are traces of similar spectra, but they are much obscured by the presence of oxyhæmoglobin. The spectrum of the kidney is worthy of notice.

In *Salamandra maculosa* the histohæmatin spectra in the kidney, liver, stomach-wall, and spleen are much obscured by the presence of hæmoglobin, but there is distinct evidence of their presence.

The Mexican Axolotl (*Siredon pisciformis*) presents well-marked spectra belonging to this class. Thus the liver shows a faint band at D, and another faint one between D and E, besides a third nearer violet, spectrum 3, Chart II. In the cortex of the kidney a well-marked histohæmatin spectrum (spectrum 4, Chart II.), which recalls myohæmatin to mind. In the stomach-wall another even more closely resembling that of myohæmatin, and in the intestine a similar spectrum (spectrum 5, Chart II.).

In the so-called red frog (species ?) the appearances are similar, the kidney showing the same double band between D and E as in Axolotl, &c. In the liver and spleen a histohæmatin spectrum like that of *Rana* occurs. The stomach-wall shows a similar spectrum to that of the kidney, especially after immersion in weak reducing agents, such as water, to which a few drops of sulphide of ammonium have been added.

In all these Amphibians myohæmatin is found.

* From the tench.

Reptiles.

In *Tropidonotus natrix* the liver, spleen, kidney, and wall of intestine show the histohæmatin bands. In the testis they also appear to be present. In a small black snake (species ? probably *Bascanium constrictor*) the liver and kidney showed histohæmatin spectra, but in the stomach-wall and intestine only traces of oxyhæmoglobin could be made out.

A small mud turtle, belonging to the genus *Trionyx*, showed in its liver, spleen, and especially in the kidney (spectrum 6, Chart II.), the usual histohæmatin spectra. In the stomach-wall only oxyhæmoglobin could be seen. In *Emys Europea* the liver and spleen showed a faint band at D, and another between D and E exactly resembling those of other animals. In the kidney a double band, as in the last case, was just visible. In the stomach-wall and that of the intestine the bands were remarkably distinct and resemble those of myohæmatin (spectrum 7, Chart II.). I found that if the portions of these organs, *e.g.*, spleen, liver, stomach, and intestine were steeped in STOKES'S fluid, the bands of the histohæmatin spectrum became much better marked. On exposure to air they became fainter; hence the banded spectrum belongs to the reduced state, and the bandless to the oxidized, and this was found to be the rule in other cases.*

In *Lacerta viridis* these spectra are beautifully marked, especially in the stomach-wall and kidney, the sharpness of the bands being much increased by steeping in glycerin. In the cortex of the kidney there is a band before D, and one, or more generally two, occupying the position of the single band, and placed closely together between D and E (spectrum 8, Chart II.). In the stomach-wall the spectrum closely imitates that of myohæmatin (spectrum 9, Chart II.), and in the intestine a somewhat similar spectrum is seen. In both these parts marked intensification of the bands is produced by dipping them into ammonium sulphide diluted with water. In the liver the spectrum is remarkable for the presence of a darker band than usual at the blue end of green in addition to those of a histohæmatin (spectrum 10, Chart II.).

In *Lacerta agilis* the same spectra are seen, also in *Scincus officinalis*.

In all the above Reptiles myohæmatin has been found.

Birds.

The common rock pigeon, *Columba livia*, furnishes evidence of the presence of histohæmatins, differing in no way from those already described. In the spleen, pancreas, liver, and kidney, the bands are well marked, especially in the last (spectrum 11, Chart II.). In the liver I have also detected free hæmochromogen. The gizzard

* Another point about the histohæmatin spectra is that they become much more distinct after the organ or tissue has been steeped in glycerin for some hours. I think this is probably due to shrinkage and the consequently greater condensation of the pigments in a given space, and to the clearing action of the glycerin.

appears mainly, if not altogether, coloured by hæmoglobin, at the same time the second oxyhæmoglobin band appeared much too dark, and was therefore probably intensified by the presence of another band belonging to a histohæmatin.*

These spectra are better marked in the snowy owl (*Nictea nivea*); in this species the cortical and medullary portions of the kidney give a beautifully-marked histohæmatin spectrum (spectrum 12, Chart II.). The liver and spleen furnish the same spectra seen in other species :—a feeble band at D, and a darker between D and E.

The stomach-wall contains oxyhæmoglobin mainly, and this result is interesting when compared with the result of examining the gizzard of other birds.

The pancreas contains a histohæmatin. In the lung oxyhæmoglobin only could be detected, and this was the case in all vertebrates examined, although not previously mentioned.

At the suggestion of Professor MOSELEY I examined the gizzards of several birds, and found generally only oxyhæmoglobin ; but in many cases I noticed the darkening of its second band as referred to above.

Other birds, *e.g.*, fowls, swifts, sparrows, and owls were examined, with results the same as the above.

Mammals.

I place the greatest reliance on the results of the examination of the organs and tissues of Mammals, because I have been better able to eliminate the influence of hæmoglobin in them by injecting the blood-vessels thoroughly with salt solution ; and in the quite bloodless organs the histohæmatin spectra are well marked.†

I do not think it necessary to figure the spectra observed in each species of mammal examined, because they are all the same practically for each organ and tissue ; but describe as briefly as possible the result of the examination of each species.

In the *hedgehog* we find histohæmatin spectra in the liver, spleen, kidney, and stomach-wall, which are the same as those found in the species already described. They may also be present in other organs, but I was satisfied at finding them in those referred to.

The *guinea pig* presents well-marked spectra belonging to the same class, in the liver in which the band between D and E is very dark, probably due to the presence of free hæmochromogen ; in the spleen, in which treatment with ammonia causes the spectrum of hæmochromogen to appear, and in the kidney, where, as in the hedgehog, a double band occurs between D and E and a feeble band at D (spectrum 13, Chart II.). In the stomach-wall I only observed the bands of oxyhæmoglobin.

In the *supra renal capsule* the *medulla* shows the bands of hæmochromogen well

* See below.

† The same method was partially successful in removing the influence of hæmoglobin in Fishes, Reptiles, and Amphibians.

marked, in fact its first band is *black*. In the cortex the spectrum is mainly that of oxyhæmoglobin, while the intermediate zone gives the spectrum of a histohæmatin. These spectra are figured in spectrum 14 and spectrum 15, Chart II. The significance of these spectra will be referred to again.

In the *rat* (especially after injection of the blood-vessels with salt solution) well-marked histohæmatin spectra can be seen. The kidney (spectrum 16, Chart II.) is remarkable for the resemblance of its spectrum to that of myohæmatin, the liver shows a feeble band at D, and a darker one between D and E, the pancreas a similar spectrum to that of the cat's pancreas, the spleen, a spectrum like that of the liver, and the stomach-wall a beautifully defined spectrum, closely resembling that of the kidney (spectrum 17, Chart II.). In the wall of the intestine similar bands occur, and the adrenals show a well-marked hæmochromogen spectrum, *which is not as distinct after injection with salt solution as it is when no injection has been used.*

The *rabbit* is a suitable animal for the demonstration of these spectra. In the spleen the spectrum observed was the same as before; the liver was remarkable for its hæmochromogen-like spectrum (spectrum 1, Chart III.); and this spectrum was got from lobules which were quite free from blood. In the kidney, the band between D and E was single, and occupied approximately the position of the double band usually observed there, and was seen in both cortical and medullary parts; in other cases spectrum 3, Chart III., was seen. The adrenals gave the spectrum of hæmochromogen in the medulla, and that of a histohæmatin in the cortex (see spectrum 2, Chart III.).

In the *dog* the spectra are similar, the spleen, liver, and kidney, stomach-wall, and supra-renal body having been examined. The medulla of the adrenals showed the hæmochromogen bands, but not as distinctly as in the guinea pig and rabbit. I was unable to measure the bands of these spectra with sufficient accuracy for wave-length calculation until I had examined the blood-free organs of the cat.

In the *cat* one can see the edges of bands by bringing the portions of organ or tissue, after thoroughly injecting with salt solution, under examination before the slit of a chemical one-prism spectroscope, as they are very distinct. Thus for the stomach-wall the following measurements were made (see spectrum 4, Chart III.)—the organs being blood-free :—

1st band (before D)	λ 613 to λ 593,
2nd band . . .	λ 569 ,, λ 563,
3rd band . . .	λ 556 ,, λ 551.

A similar spectrum is yielded by the pancreas, of which the first band extends from :—

λ 613 to λ 596·5,

and the others read :—

2nd band . . .	λ 569 to λ 563,
3rd band . . .	λ 556 ,, λ 548·5,
4th band . . .	λ 532 ,, λ 513 (?).

(Chart III., spectrum 5.)

In the liver the bands read :—

1st band	. . .	λ 613 to λ 596·5,
2nd band	. . .	λ 569 ,, λ 548·5,
3rd band	. . .	λ 537 ,, λ 521·5 (?).

(Chart III., spectrum 6.)

It is worthy of notice that the second band exactly occupies the place of the second and third bands of the histohæmatin spectrum of the pancreas.

A similar spectrum is yielded by the kidney (cortex) in which the bands read :—

1st band	. . .	λ 613 to λ 596·5, or λ 593,
2nd band	. . .	λ 569 ,, λ 563,
3rd band	. . .	λ 556 ,, λ 550. (Chart III., spectrum 7.)

In the wall of the small intestine the bands are exactly the same as in that of the stomach.

In the ovary there is also a peculiar spectrum, which is like that of hæmochromogen, but the bands are much fainter than those of the medulla of the adrenals. (Spectrum 8, Chart III.)

The adrenals, after injection with salt solution, do not show the hæmochromogen spectrum nearly as distinctly as in a cat whose blood-vessels are not thus treated. This observation obviously proves that a portion of the hæmochromogen has been washed out by the injection, and that, therefore, it must be looked upon as an excretion. (See Chart III., spectrum 9.)

Comparing now the measurements of the above spectra in wave-lengths with those of myohæmatin, one cannot help concluding that these spectra belong to the same class of pigments, or indeed to the same pigment; hence these observations prove that *myohæmatin belongs to the histohæmatins;* and had my vision been more acute, I believe I could have proved the same thing for the histohæmatin spectra of all the animals examined.

Having now proved the point which has been foreshadowed by previous observations, I may refer to some other observations as briefly as possible.

Histohæmatins have been found in the mesenteric lymphatics of the *pig*, and in the kidney, liver, and spleen of the same animal, and hæmochromogen in its adrenals; in the liver, kidney, spleen, and mesenteric glands of the *ox* and *sheep* and hæmochromogen in their adrenals.

In *man* I failed to find a histohæmatin spectrum in the thyroid, the spectrum being that of oxyhæmoglobin; the same remark applies to the pituitary body. In the thymus of a child, aged 10 months, I perceived a faint histohæmatin spectrum, but not in its thyroid. In the spleen, liver, and kidney of man, the spectra are the same as in other Mammals, and in the human adrenals hæmochromogen can be detected in the medullary part, while the cortex furnishes evidence of the presence of a histohæ-

matin. To be quite certain as to the exact spectra of the histohæmatins in man, injection of the blood-vessels with salt solution would be required, and until this has been done I do not feel warranted in drawing conclusions from other observations which I have made on the human organs. In man, however, the presence of myohæmatin has been detected, as will be described further on, as well as its occurrence in the other Mammals referred to above.

In nervous tissues* I have not yet found a histohæmatin, either in invertebrates or vertebrates.

In no animal have I succeeded as yet in isolating the histohæmatins. In them the coloured constituent occurs united to a proteid in all probability, and hence the difficulty attending attempts at isolation. Bisulphide of carbon, chloroform, alcohol, ether, benzol, petroleum, hot and cold water, alkaline water, acidulated water, and other solvents have been tried in vain.

On the other hand, oxidation and reduction can be brought about in the *solid* organs and tissues. An interesting point bearing upon this is that the bands of the spectrum of the histohæmatin in the stomach of the rat are invisible if the animal be killed during digestion, but bits of the stomach-wall plunged into a reducing agent, show in a short time the banded spectrum; the reason of this is that the histohæmatin is charged with oxygen during digestion as denoted by its bandless condition. At all events, I have proved to my own satisfaction that the *banded condition belongs to the reduced state, and the bandless to the oxidised;* but it would appear from what I have to relate about myohæmatin that this oxidation and reduction are not as simple as the reduction and oxidation of hæmoglobin, for example, the oxygen being apparently more firmly fixed than in the case of oxyhæmoglobin.

Thus, from Echinoderms† to man throughout the animal kingdom, we find in various tissues and organs a class of pigments whose spectra show a most remarkable resemblance to each other; they are allied to hæmochromogen, the bands of which are sometimes closely imitated by the histohæmatins. They are probably simpler in constitution than hæmochromogen prepared from vertebrate blood, at least they do not yield all its decomposition products; their bands are intensified by alkalies and enfeebled by acids, intensified by reducing agents, and enfeebled by oxidising agents; they accordingly appear to be capable of oxidation and reduction and are therefore *respiratory.* If this view be correct, and I have every reason to believe that it is, we may consider that the histohæmatins are of use in enabling the tissues in which they occur to take up the oxygen from the circulating blood and *hold it* in the tissues, exchanging for it the carbon dioxide. Hence the histohæmatins are concerned in the *internal* respiration of the tissues and organs of invertebrate and vertebrate animals. Why a

* The occurrence of hæmoglobin in the ventral chain of ganglia of *Aphrodite* (LANKESTER) is worthy of notice.

† The pigments of *Actiniæ* which replace (in them) the histohæmatins have been described in my paper on the "Chromatology of Actiniæ," Phil. Trans., vol. 176, p. 641.

coloured constituent should be more useful than a *colourless* one is not clear, but in hæmoglobin, hæmocyanin, and my echinochrome and Professor LANKESTER's chlorocruorin, as well as probably in SORBY's aphidein, we have colouring matters which are *respiratory pigments.*

II. MYOHÆMATIN.

Until I had discovered the colouring matter which I have named myohæmatin, it was believed by all physiologists that hæmoglobin was the colouring matter to which the voluntary and cardiac muscles of animals owe their colour. The diaphragm of the rabbit after injection with salt solution being the muscle which has been used to demonstrate the presence of hæmoglobin (KÜHNE*). While there is no doubt about the presence of hæmoglobin in the plasma of the muscle of the heart, and in that of the voluntary muscles of most vertebrates, as KÜHNE and others have shown, or in the muscular fibres of certain gasteropod molluscs, as Professor LANKESTER has shown, there is convincing evidence of the presence of myohæmatin in the cardiac and voluntary muscles of invertebrates and vertebrates, which myohæmatin either accompanies, replaces, or is replaced by hæmoglobin in individual cases. I will now give an account of the observations by which I have been led to this conclusion. I may, however, state here that Professor KÜHNE has informed me that he has long "suspected" the presence of a yellow colouring matter in muscle.†

Myohæmatin in Arthropods.

It is now about two and a half years since I discovered a new spectrum in the muscles of *Hydrophilus* and *Dyticus*. In these beetles the muscles removed from the thorax, which have a reddish-yellow, or yellow colour, give a beautifully defined spectrum which is remarkable, in the first place, for the narrowness of its bands, and, in the second, from the fact that they differ totally from the bands of any decomposition product of hæmoglobin. Neither acid nor alkaline hæmatin, acid or alkaline hæmatoporphyrin, nor methæmoglobin, nor hæmochromogen, has the remotest resemblance to this pigment with regard to the spectrum. I next found this spectrum in the alar muscles of every insect which I examined, and afterwards in the voluntary and heart muscles of every vertebrate, it was then systematically traced throughout the whole animal kingdom.

To study the spectrum of myohæmatin uninfluenced by the presence of other pigments one has only to open the thorax of the meat-fly (*Musca vomitoria*), place the reddish yellow muscle in the compressorium, throw a good light through the object and examine with the miscrospectroscope. On doing this, three bands, two of which are of great narrowness, and beautifully sharp, are seen ; the first is placed before the

* *Cf.* GAMGEE's 'Physiological Chemistry,' and KÜHNE's 'Lehrbuch der physiologischen Chemie.'

† It is more correct to describe it as yellowish-red, and a concentrated solution, obtained by a method which I hope to describe shortly, is a *red* colour.

FRAUNHOFER line D, two others narrow and sharp, of which the second is darker than the first, between D and E, and generally two others nearer the violet (spectrum 10, Chart III.). The first three in the case of *Geotrupes stercorarius* read :—

1st band . . .	λ 613	to λ 593,
2nd band . . .	λ 567·5	„ λ 561·5,
3rd band . . .	λ 554·5	„ λ 546,
4th band . . .	λ 532	„ λ 511·5 (about).

In the alar and other voluntary muscles of other insects (and I have now examined a great number) myohæmatin is the pigment to which they owe their reddish-yellow or yellow colour. I do not think it necessary to give a drawing of the spectrum of myohæmatin in each case, as its bands always occur in the same part of the spectrum; besides anyone who possesses a microspectroscope can easily verify the truth of my statements for himself.

In *Hydrophilus piceus* and *Dyticus marginalis*, as already stated, the myohæmatin bands are seen with great distinctness, and in *Hydrophilus* early in 1883 I measured the bands in wave-lengths with the following result :—

1st band . . .	λ 613 to λ 593		
2nd band . . .	λ 569 „ λ 563		Approximate.
3rd band . . .	λ 557 „ λ 548·5		

The other bands could not be accurately determined. By heating this muscle in rectified spirit and caustic potash I obtained a small quantity of a yellow solution showing some feeble shading in green, and on adding sulphide of ammonium two bands like those of hæmochromogen came into view, but much nearer violet than those of hæmochromogen, thus the first extended from about λ 557 to λ 548·5, and evidently corresponded to the third band seen in the muscle. I would call attention to this fact, because a similar spectrum is seen in some insect muscles without any treatment, and it was also seen in a solution of isolated myohæmatin from vertebrates obtained by digesting the blood-free muscle in pepsine and water.* This kind of modified myohæmatin was met with in *Lucanus cervus*, as shown in spectrum 11, Chart III., also in some specimens of *Periplaneta orientalis*, in *Bombus terrestris*, and in *Apis mellifica*, in all of which species the unmodified myohæmatin also is found. In *Lucanus* these bands of changed myohæmatin read :—

1st band . . .	λ 557	to λ 548·5,
2nd band . . .	λ 532	„ λ 516 (?).

and therefore are placed in the same part of the spectrum as the third and fourth (?) bands of the normal myohæmatin spectrum.

* See Chart IV., and *cf.* with spectrum of muscle of *Lucanus*, &c.

In *Cerambyx moschatus* the bands of myohæmatin became much more distinct after they had been treated with ammonium sulphide, and then read :—

1st band	. . .	λ 613 to λ 593,
2nd band	. . .	λ 570·5 ,, λ 564·5, (?)
3rd band	. . .	λ 558·5 ,, λ 551 (?).*

I noticed that the spectrum of these muscles became much feebler on exposure to air.

In *Creophilus maxillosus* the same four bands were well marked (spectrum 12, Chart III.).

In *Carabus violaceus*, of which a great number have been examined, the spectrum is equally satisfactory.

Although the muscle in *Coccinella bipunctata* is small in amount, yet the same spectrum was seen in it.

In *Periplaneta orientalis* we sometimes meet with the modified variety of myohæmatin of which the spectrum representing that of *Lucanus* is the same as in this, while in the bulk of the specimens examined the four-banded myohæmatin is present.

In *Staphylinus olens* whose testes, as already said, are coloured by hæmochromogen and possibly hæmoglobin, both the thoracic and leg muscles yield the spectrum of myohæmatin only.

Geotrupes stercorarius shows the myohæmatin spectrum in the thoracic and leg muscles beautifully marked. Spectrum 13, Chart III.

The wave-length measurements have been given above.

In *Gryllus domesticus* the thoracic muscle yields the same spectrum, and in the large thigh muscles it is well marked.

In dipterous, hymenopterous, and lepidopterous insects, those which use their muscles actively in moving the wings have the greatest amount of myohæmatin.

In *Tipula oleracea* although its thoracic muscle is small, it shows a beautifully defined myohæmatin spectrum; *Musca domestica*, *M. vomitoria*, and *M. chlora* are equally satisfactory; thus the alar muscle of a blue-bottle fly gave spectrum 10, Chart III.

In *Apis mellifica*, *Bombus terrestris*, and *Vespa vulgaris* myohæmatin is present abundantly. The alar muscle of *Bombus* sometimes shows the modified myohæmatin spectrum of which the bands in this case read :—

1st band	. . .	λ 556 to λ 550,
2nd band	. . .	λ 532 ,, λ 513 (?).

In other specimens the normal spectrum was seen. (Spectra 14 and 15, Chart III.) The same remark applies to *Apis*.

* Although (?) are placed after these it will be seen that they correspond closely enough with other measurements.

In those muscles of *Bombus* which contained the modified myohæmatin, treatment with ammonium sulphide developed the second myohæmatin band, but that before D could not be seen, *i.e.*, all the bands of the four-banded spectrum were present except the first. After the muscles of a moth had stood some time in glycerin the change into modified myohæmatin also took place, and on treating with ammonium sulphide the above-described change took place.

In *Acrida viridissima* I found myohæmatin present, but as in other orthopterous insects it is not present as abundantly as in dipters and lepidopters. On treatment with sulphide of ammonium the bands become much more distinct. (For *Periplaneta* and *Gryllus* see above.)

The diurnal and nocturnal lepidoptera show abundance of myohæmatin in their alar muscles, thus in *Pieris rapæ*, and other butterflies of which several have been examined, the spectrum is exactly the same as that described above. In various night moths, which need not be enumerated, it is present in abundance, and it also occurs in the mouth parts of various larvæ.

I have tried to isolate myohæmatin from insect muscle but without result, owing to dearth of material. After boiling in water, the solution appeared to have a faint bluish fluorescence, due probably to the presence of fine particles in suspension; and the muscle itself no longer showed the original spectrum but that of modified myohæmatin described above (except that the original spectrum was not restored with NH_4HS). The action of various solvents was tried in vain.

Acids cause the bands to disappear. Caustic alkalies (in strong solution) modify the spectrum when added to the solid muscle, the band before D disappears, another narrow band appears after D, and the dominant (3rd) band changes, being now made up of a lighter part nearer red, and a darker nearer violet; on treatment of the muscle (thus treated) with ammonium sulphide no noticeable change takes place. On treating the muscle of a moth with acetic acid the bands disappear, and on again adding caustic soda the bands appear as they do when caustic soda alone is used. With hydrochloric acid the bands also disappear, and on again adding caustic soda the third and fourth bands of the normal myohæmatin spectrum reappear, the spectrum thus reproduced resembling the modified spectrum described above.

When an attempt is made to mount an insect muscle in Canada balsam, the preliminary treatment destroys the bands, they are not much changed by being placed in alcohol, but in oil of cloves the bands completely disappear; this change is no doubt due to the ozonising action of the oil of cloves.*

On treating a muscle from a moth's thorax with peroxide of hydrogen the bands disappear, and the muscle is bleached by this treatment. On again treating with a reducing agent (*e.g.*, sulphide of ammonium) a new spectrum appears, the second and

* In glycerin, specimens of muscle preserve the banded spectrum of myohæmatin for months, but it finally becomes altered into the modified variety. Insect muscle mounted in glycerin jelly soon changes as to the spectrum.

third myohæmatin bands having partially coalesced, and the second band being as dark as the third, the band before D being, however, well marked. Hence this change is not the same as that effected by acids. I have tried the effect of a current of oxygen passed over insect muscle placed in a suitable gas chamber placed beneath the micro-spectroscope; but, although the gas was passed for ten minutes, and the experiment several times repeated, the myohæmatin bands persisted. The reason, of course, may be that the gas did not penetrate the substance of the muscle, but merely passed over its surface.* Still the fact remains that exposure to air diminishes the intensity of the bands, that as I have found repeatedly the bands are less distinct when the insect is killed in flight, that when thus invisible, or nearly so, they can be brought back by the use of STOKES's fluid or ammonium sulphide. Hence, then, myohæmatin can be oxidised and reduced in the case of insects; its fully oxidised state is denoted by the absence of bands, its reduced state by their presence. The same was found to be the case with the histohæmatins, so that one may safely conclude that *these pigments are concerned in the respiration of the tissues in which they occur.*

Myohæmatin also occurs in spiders. It is present in the muscle of the cephalo-thorax of *Epeira diadema, Tegenaria civilis,* and other spiders; in these the muscles are pale, and, therefore, contain but little myohæmatin, but its bands can generally be brought into view by treatment with reducing agents (spectrum 16, Chart III.).

In *Crustaceans* the distribution of myohæmatin is confined apparently to the cardiac muscle.

In all the specimens of *Astacus fluviatilis* examined the heart muscle showed the spectrum of myohæmatin, all five bands being visible. In the voluntary muscle it could not be found (spectrum 17, Chart III.).

In the heart of *Cancer pagurus* it is found, and it is absent from the other muscles (spectrum 18, Chart III.).

In the heart of *Homarus vulgaris* the myohæmatin bands could be measured in the large spectroscope and gave the following readings:—

1st band . . . λ 613 to λ 593,
2nd band . . . λ 569 ,, λ 563 (about),
3rd band . . . λ 556 ,, λ 550 (spectrum 1, Chart IV.).

It was absent in the voluntary muscle.

In *Pagurus bernhardus* it is present in the heart muscle, but as in the others absent from the voluntary muscle.

In *Carcinus mœnas* the heart muscle† shows myohæmatin bands.

In all these cases the bands are made more distinct by the use of reducing agents and fainter by exposure to the air.

* It is well known that the O in the O-hæmoglobin of vertebrate muscle is not given off *in vacuo.*

† But *not* the voluntary muscles.

Myohæmatin in Mollusca.

It occurs in all the pulmonates examined. Thus in *Limax flavus* the heart muscle shows it well; it is present more abundantly in the ventricle than in the auricle. It is present in the pharyngeal muscle of the same species (spectrum 2, 3, Chart IV.).

In *Arion ater* it is present both in the cardiac and pharyngeal muscle (spectrum 4, Chart IV.).

Limax maximus presents a beautiful myohæmatin spectrum in the ventricle of the heart, also in the pharyngeal muscle. In the heart and buccal muscles of *Helix aspersa* the myohæmatin bands are well marked (spectrum 5, Chart IV.). In the microspectroscope, as the drawings show, the bands are coincident with those of the myohæmatin of other species; owing to the smallness of the heart I could not measure these bands in wave-lengths, but there is no doubt that the measurements are the same. It also occurs in the heart and pharyngeal muscles of *Helix pomatia.*

*Myohæmatin in Vertebrates.**

The myohæmatin of vertebrates differs in no essential respect from that of invertebrates. In some cases the band before D is slightly narrower, or may sometimes (exceptionally) be double. The dominant bands are always the same.

Fishes.

In the mackerel (*Scomber scombrus*) myohæmatin occurs in the heart; it is well marked in the ventricle. In the pale dorsal muscles its presence is uncertain, in those along the lateral line, whose colour is brownish-red, it occurs mixed with some oxyhæmoglobin; in other parts of the lateral muscle it is well marked. (Chart IV., spectrum 6.)

In the herring (*Clupea heringus*) it is found in the ventricle (mixed with oxyhæmoglobin as in the mackerel). Its first band being somewhat narrower than usual; also in the auricle. It is mixed with oxyhæmoglobin in the more reddish parts of the dorsal muscles, and in the pale abdominal muscles it presence is uncertain.

In the roach (*Leuciscus rutilus*) it occurs in the ventricle of the heart mixed with oxyhæmoglobin, also in the muscle close to the lateral line; in the paler dorsal muscles its presence is uncertain, as is that of oxyhæmoglobin.

In the tench (*Tinca vulgaris*) it also occurs in the ventricle of heart mixed with oxyhæmoglobin. The dorsal (pale) muscle showed only traces of oxyhæmoglobin, the redder muscle along lateral line showed the same with traces of myohæmatin. In the tongue muscle only oxyhæmoglobin could be detected.

It has also been found in the heart muscle of the *plaice, eel, codfish, salmon, whiting, goldfish,* and others.

* I have not thought it necessary to map the bands from the voluntary muscles as they are the same as in the heart of each animal examined.

Amphibians.

In *Bufo vulgaris* myohæmatin occurs in the heart, and in the voluntary muscles mixed with oxyhæmoglobin, it is well marked in those of the thigh. (Spectrum 8, Chart IV.)

In *Rana temporaria* it occurs well marked in the ventricle and feebly in the auricle, in both places mixed with oxyhæmoglobin. In faint traces in the thigh and calf muscles, which are very pale. Also in other muscles. (See spectrum 7, Chart IV.)

In *Salamandra maculosa* it is present mixed with oxyhæmoglobin in the ventricle; in traces in the arm and leg muscles and elsewhere in the voluntary muscles.

In the little green tree-frog (*Hyla arborea*) the distribution of myohæmatin was more carefully studied, and here I may observe that its absence must not be concluded from the fact that the muscle does not show the bands at once. If present in the oxidised state they may be invisible, so that a reducing agent may be necessary to bring them out. In most cases I used sulphide of ammonium in water or STOKES's fluid. It is present in *Hyla's* ventricle and in the auricle, in the flexors of thigh, and in its extensors; in the tongue muscles in traces; in the calf muscles; in the muscles in anterior aspect of leg; in the muscles of arm and forearm, and in all these apparently it is the sole pigment. It is mixed with oxyhæmoglobin in the muscles of the back and sometimes in those of tongue.

In the Axolotl (*Siredon pisciformis*) a feeble myohæmatin spectrum was seen in the ventricle and auricles, faint traces in the abdominal muscles, in those of the tail, and in the muscles of the leg; while in those of the tongue, and of the back only oxyhæmoglobin could be seen. In the "*red frog*" (species ?) myohæmatin occurs in the ventricle and in various voluntary muscles, *e.g.*, pectorals, muscles of thigh, of leg, and it was generally found mixed with oxyhæmoglobin.

Reptiles.

In a small mud turtle of the genus *Trionyx* the heart ventricle showed the myohæmatin bands so distinctly that I was able to measure them (approximately):—

1st band . . . λ 613 to 595,
2nd band . . . λ 569 „ 563,
3rd band . . . λ 556 „ 550. (See spectrum 9, Chart IV.)

The same bands are well marked in the pectoral muscles; and in those of leg and arm feebly.

In *Lacerta viridis* the same bands are well marked in the ventricle, and measured as follows:—

1st band . . . λ 613 to λ 593 (or λ 595),
2nd band . . . λ 569 „ λ 563,
3rd band . . . λ 556 „ λ 550,
4th band . . . λ 532 „ λ 513 (approximate).

In the flexor and extensor muscles of thigh, I could only find oxyhæmoglobin, in a muscle in front of pubis myohæmatin, in flexor muscles of forearm myohæmatin, in biceps and pectorals myohæmatin and oxyhæmoglobin, in calf muscles traces of myohæmatin, in those of tail only oxyhæmoglobin, in triceps and other extensors of arm myohæmatin and oxyhæmoglobin, in dorsal muscles only oxyhæmoglobin, and in depressors of mandible oxyhæmoglobin. In a second specimen the distribution was not quite the same, thus the tail muscles showed myohæmatin, the flexor muscles of arm myohæmatin and traces of oxyhæmoglobin, the same in its extensors; in flexors of thigh myohæmatin and oxyhæmoglobin, anterior muscle of leg the same, in calf muscles myohæmatin and oxyhæmoglobin, in mylohyoid both pigments, in thoracic intercostals only oxyhæmoglobin, in dorsal muscles both pigments, in tail muscles both pigments. This last lizard had its vessels injected with salt solution which partially accounts for the differences noted. (See spectrum 10, Chart IV.)

In *Emys Europæa* myohæmatin was found in auricle and ventricle, in the pectorals, in dorsal muscles traces mixed with oxyhæmoglobin, in tail muscles oxyhæmoglobin, the same in pelvic muscles, both pigments in the flexors and extensors of arm, and in the flexors and and extensors of leg.

In *Lacerta agilis* myohæmatin is well marked in the ventricular muscle, and in the biceps of arm; the redder parts of the flexors of forearm show the presence of oxyhæmoglobin, the paler myohæmatin; in the extensors only myohæmatin, the pectorals myohæmatin, in flexors of leg myohæmatin, the same in calf muscles, pelvic muscles traces of the same, muscles of tail oxyhæmoglobin and myohæmatin, dorsal muscles traces of myohæmatin, mylohyoid myohæmatin, and this pigment occurs well marked in the tongue muscles.

In *Scincus officinalis* myohæmatin is present in auricle and ventricle; and in the pectorals mixed with oxyhæmoglobin, in the triceps both pigments occur, in the tail muscles oxyhæmoglobin principally. In other muscles the distribution is much the same as in the above species. In *Tropidonotus natrix* the myohæmatin bands of auricles and ventricles give the following readings:—

1st band . . .	λ 613	to	λ 593,	
2nd band . . .	λ 569	,,	λ 563,	
3rd band . . .	λ 556	,,	λ 550,	
4th band . . .	λ 532	,,	λ 513.	(?)

The same bands are well marked in the tongue muscles; in the mylohyoid and other muscles of mandible myohæmatin and oxyhæmoglobin are found, the latter, too, principally in the dorsal muscles and intercostals.

In a black snake (*Bascanium constrictor ?*) the same bands can be seen in the ventricle and auricle, in the muscles along the body both pigments occur, also in the depressors of the lower mandible, in the dorsal muscles, and in the intercostals. The bands of myohæmatin are badly marked in all these voluntary muscles.

Birds.

So far there is no difference in the spectrum of myohæmatin in all the animals examined. In birds and mammals the band at D may vary slightly, as will be seen when the spectra are described.

In the pigeon (*Columba livia*), while the dominant bands of myohæmatin are present in the ventricle of the heart that at D may be replaced by two ; the same bands are seen in the auricle. In the pectorals and some leg muscles the band at D is the same as in the above-mentioned species; and in other voluntary muscles—where myohæmatin occurs generally with oxyhæmoglobin—this band is also similar.

In the snowy owl (*Nictea nivea*) the ventricle shows the usual myohæmatin spectrum (spectrum 11, Chart IV.), also the auricle. It is present abundantly in the pectoral muscles (as it is in all birds),* sparingly in the leg muscles, being apparently replaced to a great extent by oxyhæmoglobin.

In the *gizzards* of fowls I have found oxyhæmoglobin, whose second band is, however, slightly darker than it should be, probably from the presence of a trace of myohæmatin.

In the heart of the *common fowl* the band at D shows frequently the peculiarity noted above in the pigeon. Here the myohæmatin bands may be measured, and give the following result :—

1st band . . .	λ 613 to λ 596·5, and shading λ 589 to λ 582,
2nd band . . .	λ 569 ,, λ 563,
3rd band . . .	λ 556 ,, λ 550 (approximate),

and these are well seen in auricle and ventricle.

In the ventricle of the *turkey's* heart the bands read :—

1st band . . .	λ 613 to λ 593 (and shading ?),
2nd band . . .	λ 569 ,, λ 563,
3rd band . . .	λ 556 ,, λ 550.

In the auricle oxyhæmoglobin appears to be the dominant pigment.

In the auricle and ventricle of the *goose* the bands read :—

1st band . . .	λ 613 to λ 593,
2nd band . . .	λ 569 ,, λ 563,
3rd band . . .	λ 556 ,, λ 550.

There are also two other bands nearer violet, too faint to be read. In the auricle and ventricle of the *duck* they give the same readings.

Putting M for myohæmatin and H for hæmoglobin, the following rough sketch will give an idea of the distribution of myohæmatin in the voluntary muscles of a bird (pigeon) :—

* In fact the pectorals of the pigeon are mainly, if not altogether, coloured by myohæmatin.

Biceps of forelimb, M and H ; triceps, M and H ; latissimus dorsi, M and traces of H ; trapezius, M and H ; muscles connecting skull to spine, M and H ; tail muscles, H ; flexors of forearm, M and H ; extensors, M and H ; sartorius (?), M and H ; other thigh muscles, M and H ; tibialis anticus, M and H ; gastrocnemius, M ; abdominal muscles, M and H ; so that in all the voluntary muscles examined, we find myohæmatin, except in those of tail.

In the gizzard of the same bird there are also decided traces of myohæmatin, but oxyhæmoglobin is the pigment to which it mainly owes its red colour.

I have also examined other birds, and in all of them the results were the same as those described.

Mammals.

In the *hedgehog's* heart,—auricle, ventricle, and auricular appendix, all give the spectrum of myohæmatin. It also is found in most, if not all, the voluntary muscles mixed with oxyhæmoglobin. The same is the case in the *guinea-pig*, and in the *rabbit* (spectrum 12, Chart IV.) ; from an inspection of this spectrum it is seen that the bands are the same as those of myohæmatin found elsewhere, and give the same readings. The following will show the distribution of myohæmatin in the voluntary muscles of a rabbit ; (putting H=Hæmoglobin and M=Myohæmatin) :— Diaphragm principally H ; pectoralis major, H and traces of M ; sub-scapularis, H ; inter-costals, H ; sterno-mastoid, M and H ; rectus abdominis, H ; obliquus externus, H ; latissimus dorsi, H ; supra-spinatus, H and M ; infra-spinatus, H and M ; masseter, M and H ; biceps of arm, M and H ; triceps of arm, M and H ; extensors of forearm, M and H ; flexors of forearm, M and traces of H ; psoas magnus, H ; gluteus maximus, H and traces of M ; rectus femoris, M and H ; an adductor of thigh, H ; vastus externus, M and H ; gracilis, H ; vastus internus, M and H ; tibialis anticus, M and H ; gastrocnemius (external head), M, and (internal), H ; flexor communis digitorum, traces of M and H ; other flexors of leg, ditto.

In the heart of the *hare* the band of myohæmatin at D of the normal spectrum is replaced by two (spectrum 13, Chart IV.), and these bands with the others gave approximately the following readings :—

1st band . . .	λ 613 to λ 600, and shading λ 593 to λ 582.	(?)
2nd band . . .	λ 569 ,, λ 563,	
3rd band . . .	λ 556 ,, λ 550.	

Here the inter-auricular septum gives both myohæmatin and oxyhæmoglobin, so does the auricular wall and appendix. In the ventricle, auricle, and auricular appendix of the *rat*, and in its voluntary muscles, the former pigment is found abundantly ; in the ventricle the bands read (Chart IV., spectrum 15) :—

1st band . . .	λ 613 to λ 596·5,
2nd band . . .	λ 569 ,, λ 563,
3rd band . . .	λ 556 ,, λ 550.

In the *mouse* the bands are the same.

In the ventricle of the heart of the *dog*, the myohæmatin bands read (Chart IV., spectrum 14) :—

1st band	. . .	λ 613 to λ 598,
2nd band	. . .	λ 569 ,, λ 563,
3rd band	. . .	λ 556 ,, λ 550.

It is also present in the wall of auricle and auricular appendix, and in the general muscular system.

In the same parts of the *cat* it is found abundantly ; in the heart ventricle, after injection with salt solution, its bands read :—

1st band	. . .	λ 613 to λ 596·5,
2nd band	. . .	λ 569 ,, λ 563,
3rd band	. . .	λ 556 ,, λ 550.

In the *sheep*, *ox*, and *pig* it also occurs in the same situations, and, lastly, it has been found in the heart and voluntary muscles of man. Its bands are seen with great distinctness in the musculi papillares of the human heart, and here its first band is narrower than usual, as shown in spectrum 16, Chart IV.

Attempts to isolate Myohæmatin.

In myohæmatin the coloured constituent is united to a proteid as in hæmoglobin and enterohæmatin, hence the difficulty which attends its isolation. I have already described some experiments on the myohæmatin of insects ; on that of mammals a few experiments have also been made, but much yet remains to be done.

It was necessary to endeavour to find out what relationship it bears to muscle plasma, as KÜHNE * has shown that the hæmoglobin of muscle belongs to the plasma. Accordingly this experiment was performed : a rabbit was killed, the vessels at the base of the heart ligatured quickly, and both ventricles injected and well washed out with salt solution ; they were then quickly cut off at their junction with the auricles ; and after cutting them up, well washed with salt solution and put into a rag and frozen in a platinum vessel surrounded with a mixture of ice and salt ; when frozen they were removed and cut with an ice-cold knife, and pressure was then brought to bear on the mass in the rag ; a few drops of a reddish-yellow liquid exuded which was examined with the spectroscope. It showed the bands of oxyhæmoglobin which appeared to me slightly changed from the normal as the second one seemed too dark, and on adding sulphide of ammonium this idea was found to be correct, as now the dominant myohæmatin bands appeared in the usual position.

In some filtrate thus obtained, just above a freezing temperature spectrum 17, Chart IV., appeared after adding some sulphide of ammonium. Hence a part of this

* *Loc. cit.*

pigment occurs in the plasma like hæmoglobin. It is noticeable that its bands are merged into those of oxyhæmoglobin, which accounts for their having been missed by others; through the thin hazy band of reduced hæmoglobin they are, however, readily seen. I missed seeing the band at D, probably owing to the small amount of plasma at my disposal. I failed to get the pigmented part of myohæmatin by the use of any solvent into solution, thus the great pectoral muscle of a pigeon, which had been injected with salt solution, was cut up into small pieces, washed with salt solution and then strongly pressed; after a second washing and pressing it was divided and portions put into—(1) an aqueous solution of caustic soda; (2) a rectified spirit solution of caustic soda; (3) a rectified spirit solution with sulphuric acid; (4) amyl alcohol.

In the first solution I could find only hæmochromogen by the use of sulphide of ammonium; in the second a trace of changed myohæmatin by using the same reagent; in the third only acid hæmatin from the muscle hæmoglobin; and in the fourth a trace of lutein (= a lipochrome).

By boiling the same muscle in rectified spirit and sulphuric acid a spectrum was obtained which was a mixture of acid hæmatoporphyrin with a decomposition product of myohæmatin. The purple-red solution giving five bands, four of which read as follows:—

1st band . . .	λ 640 to λ 623,	
2nd band . . .	λ 605 ,, λ 594,	(or 3)
3rd band . . .	λ 582 ,, λ 576,	(?)
4th band . . .	λ 563 ,, λ 545·5.	

By similar *heating* with caustic soda and rectified spirit only hæmochromogen could be detected in the solution by adding ammonium sulphide.

(5) Ether failed to take up any pigment, also (6) benzol, but (7) chloroform took up a trace of a pigment, the solution being faintly yellow and showing a very narrow band in green and two belonging to lutein.

By the use of pepsine I succeeded in isolating a *changed* pigment. Some of the blood-free muscle from the ventricle of the heart of a sheep was cut up small, and after steeping in water for twelve hours, strongly pressed in a linen rag. This was now digested in a solution containing 0·6 per cent. oxalic acid (KÜHNE) and a little pepsine for two hours at a temperature of 98° F. This solution, after filtering, showed two bands having some resemblance to hæmochromogen, but placed nearer violet. They are evidently similar bands to those seen as an exception in the thoracic muscle of the bee and the cockroach, &c., *supra;* they read:—

1st band . . .	λ 554·5 to λ 548·5,
2nd band . . .	λ 524·5 ,, λ 519* (=narrow part).

On adding some caustic soda the bands disappeared, but with ammonium sulphide

* This second band does *not*, however, correspond to that of altered myohæmatin.

they reappeared. Acetic acid did not seem to affect them. On examining the muscle thus treated I could still see a trace of the band before D, but the others had disappeared.

Some of the filtered pepsine extract was evaporated to dryness on the water-bath and left a *yellowish-red,* amorphous residue. This was insoluble in alcohol or chloroform (which each removed a trace of lutein, and other matters, but not the pigment), in benzol and light petroleum, but it easily went into *distilled water*, forming a yellow solution and giving the bands referred to above, and with sulphide of ammonium the bands were intensified (spectrum 18, Chart IV.), and read :—

1st band	. . .	λ 554·5 to λ 548·5,
2nd band	. . .	λ 524·5 ,, λ 519.

There still remained in the dish traces of a purplish-brown pigment, which was probably changed myohæmatin, from the reaction obtained with it. By the use of trypsin digestion I failed to isolate the same pigment.

I succeeded in isolating the same pigment from the pectoral muscle of a pigeon, and got the above results. Hence any attempt at isolation alters the nature of the pigment, except freezing. But we may so far conclude that myhoæmatin is a yellow or reddish-yellow pigment, and when isolated and changed soluble only in water.

I hope to continue these experiments shortly.

The Hæmochromogen of the Medulla of the Adrenals and the Pathology of ADDISON'S *Disease.*

In the adrenals, as already shown, we meet with a spectrum which is totally different from that of the histohæmatins, for while in their cortex a histohæmatin may be present, in the medulla *free* hæmochromogen occurs. A similar spectrum may be met with occasionally in the liver, and perhaps in the spleen. Its bands are certainly diminished after the injection of salt solution, hence here the hæmochromogen must be looked upon as an excretion. This spectrum is very constant, and so far I have found it in the adrenals of man, dog, cat, rabbit, rat, guinea-pig, ox, and sheep. VULPIAN has shown that taurocholic acid exists in the medulla,* and other observers have found products which indicate the existence of an active downward metabolism. Taken in connexion with my own observations these facts go to prove that the adrenals are organs which are concerned in the retrogressive metamorphosis of hæmoglobin, and probably of the pigments described in this paper—the histohæmatins and myohæmatin. Hence if the adrenals are not in a fit state for the discharge of their functions we may have pigments present in the circulation which are incompletely metabolised. Now, I have shown that such is the case, as the urine of ADDISON'S disease may contain a

* Other constituents are leucin, hypoxanthin, benzoic and hippuric acids, taurin, &c.

pigment which I formerly described under the name of "urohæmatin,"* which is a pigment closely related to hæmatoporphyrin, and which may also appear in the urine when the adrenals are healthy if there be an excessive destruction of blood-corpuscles, as in acute rheumatism; in such cases there being more effete colouring matters present than the organs concerned in their further downward metamorphosis are capable of dealing with. I need not describe the characters of urohæmatin more fully here, as I have done so elsewhere,† but merely call attention to the significance of its presence.

Recent researches‡ on the development of the adrenals go to negative the idea that they are nervous ganglia, and I believe they may be looked upon as organs which are more or less supplementary to the liver, which have been developed at a later stage (*i.e.*, a later stage in the ancestral development), when the animal body had become more complex, and a greater abundance of respiratory pigments had become necessary for internal and ordinary respiration; which entailed the setting apart of certain organs to carry on the increased "division of labour;" in this case the function of these supplementary organs being the removal from the circulation of useless and worn-out pigments, and their accompanying proteïds.

Whether this view be correct or not, it fits in well with the teachings of physiology and pathology. For TIZZONI has shown that the removal of the adrenals is followed by pigmentation of the skin and mucous membranes, and in the majority of cases of ADDISON's disease in which well-marked bronzing of the skin took place they have been found diseased. The occurrence of ADDISON's disease without apparent disease of the adrenals can be easily explained on the hypothesis that they are supplementary organs, as other organs may do duty for them, but it remains to be proved whether, when they are apparently healthy in cases of bronzing of the skin, other conditions may not be present which hinder the discharge of their functions, such as obliteration of their arterial or nervous supply.

No sufficient explanation has yet been offered as to the causation of ADDISON's disease, and if the facts described in this paper should help to solve this problem it may induce others to follow up these observations, which at first sight seemed to have but the remotest bearing on human pathology. I have tried to obtain pigments from the adrenals by the use of various solvents, but as yet without result.

Are there any grounds for supposing that the product of their metabolic activity may be derived from the histohæmatins? I believe this question may be answered in the affirmative for this reason, that in the integument of *Uraster rubens*, as already mentioned, hæmatoporphyrin occurs *as such*, and here it is highly probable that it is a

* Proc. Roy. Soc., vol. 31, p. 26, and vol. 35, p. 394; also 'Journal of Physiology,' vol. vi., pp. 22 *et seq.*

† *Loc. cit.*

‡ Compare W. F. R. WELDON "On the Supra-renal Bodies of Vertebrates," Quart. Journ. Micros. Soc., vol. xxv., pp. 137 *et seq.*; also BALFOUR's "Treatise on Comparative Embryology;" and ALLEN THOMSON in the 9th Edition of "QUAIN's Anatomy."

derivative of the histohæmatins, for in *Uraster* besides the lipochromes and enterochlorophyll, these are its only available source. This hæmatoporphyrin is nearly related to the urohæmatin found in ADDISON'S disease, as I have already stated. Hæmatoporphyrin also occurs (as I have shown) in the integument of *Limax* and *Arion*,* and in their bodies again it is probably derived from the histohæmatins and possibly from enterohæmatin. Enterohæmatin presents same remarkable resemblance to myohæmatin. (1) Neither can be converted into all the decomposition products of hæmoglobin. (2) Both are composed of a proteid and colouring matter united together. (3) In both acids cause the bands to disappear. (4) In both potassium hydroxide and sodium hydroxide intensify the bands. (5) In both the banded condition belongs to the reduced state and the bandless to the oxidised. Again myohæmatin can be changed into a body which closely resembles (in its spectrum) enterohæmatin. Hence in saying that enterohæmatin may be the mother-substance of the integumental hæmatoporphyrin, is practically the same as saying that it may be derived from the histohæmatins.

I have endeavoured to find out if anything like myohæmatin could be prepared from any of the decomposition products of hæmoglobin, but without any definite result. In a solution of alkaline hæmatoporphyrin (in rectified spirit and ammonia) a change took place spontaneously, after the solution had been shut up from air and light for some months; the four-banded spectrum disappeared and could not be regenerated, and in its place I observed some narrow bands closely resembling those of myohæmatin, but placed much nearer the red end of the spectrum. They were narrower than those of any other decomposition product of hæmoglobin, but the solubility of the pigment and its refusal to be reduced, showed that it was not myohæmatin. Acting on this hint, I tried the effect of various oxidising and reducing agents on hæmatin and hæmatoporphyrin, but no myohæmatin-like spectrum could be obtained; but it is probable that myohæmatin is much nearer to hæmoglobin than to hæmatoporphyrin, and the above may be an accidental resemblance.

The Function of the Histohæmatins and Myohæmatin.

I need not repeat the results obtained by the action of oxidising and reducing agents on these pigments, it will suffice to say that they are capable of oxidation and reduction, and are hence respiratory. Just as in *Actiniæ*—as I have shown in a former paper,—actiniohæmatin is concerned in the respiration of the tissues, so these pigments are also concerned in it. They combine with the oxygen conveyed to them in the blood, and hold it for purposes of metabolism, parting with the carbon dioxide in exchange for the oxygen. This is the only conclusion which anyone who has gone over the same ground can come to. These observations appear to me to point to the fact that the formation of CO_2 and the absorption of oxygen takes place *in the tissues* themselves (PFLÜGER and OERTMANN) and not in the blood. Hence these obser-

* Proc. Birm. Phil. Soc., vol. iii., pp. 351–407; Journ. Physiol., vol. vi., pp. 22–39, and vol. vii., pp. 240 *et seq.*

vations are of value in helping to decide a difficult point. I have not succeeded yet in finding out whether these pigments are dissolved in the plasma bathing the tissues, or in the *solid* parts of the tissues. In the case of muscle, certainly a small amount of myohæmatin was obtained from the plasma, but after squeezing out all juice, the muscular fibre showed abundance of myohæmatin, which at the same time may have been prevented exuding by coagulation of the myosin. I am inclined to believe that a certain amount of myohæmatin belongs to the *solid* (or semi-solid) part of the muscular fibre, and probably the same remark applies to muscle hæmoglobin; for after all the opposite conclusion is mainly based on KÜHNE and EBERTH's observation of the presence of a living nematode within the sarcolemma, and this does not altogether exclude the possibility of the presence of a colouring matter in the sarcous substance.

I have not insisted strongly on the fact that myohæmatin is a histohæmatin, as anyone cannot fail to come to that conclusion who studies the accompanying charts. Myohæmatin then may be considered as the true intrinsic colouring matter of muscle, and the histohæmatins the intrinsic colouring matters of the tissues and organs; both may be reinforced or replaced at times by hæmoglobin when extra activity of internal respiration is required; probably the same radical may be made use of for building up all these pigments, at all events they seem to be related, since the same decomposition product—hæmatoporphyrin—is probably yielded by all of them. The fact that in the lower animals pigments of less complex molecular structure than hæmoglobin and identical with its decomposition products can function like it, forces itself on anyone's attention who studies the pigments of the Invertebrata.

Lastly, I would call attention to another striking fact which I have found out, thanks to Professor MOSELEY. In his most valuable Paper* on "animal colouring matters," which he discovered while on board the "Challenger," he describes a madder-coloured pigment named by him polyperythrin, and he suggested that it might have some connexion with some of the pigments found by me in *Actiniæ*.† It was found by Professor MOSELEY in simple stony corals of very different genera, in two forms of Actiniæ from deep water, and in certain hydroids. In the drawing accompanying Professor MOSELEY's Paper he figures an acid and an alkaline spectrum, and it will be noticed how these spectra resemble acid and alkaline hæmatoporphyrin respectively. On examining some slides of the dried pigment kindly sent by Professor MOSELEY, I found that the bands of the neutral dried pigment corresponded very closely (although not exactly) to those seen in artificially-prepared neutral hæmatoporphyrin, and in that obtained from the integument of *Uraster rubens*, &c., and still more closely to the bands in the egg-shell of a Cochin China hen.‡ Moreover, with good illumination,

* "On the Colouring Matters of Various Animals," &c., Quart. Journ. Micros. Soc., vol. xvii., N.S., pp. 1–23.

† "Chromatology of Actiniæ," Phil. Trans., vol. 176, p. 641.

‡ In which, as in many other birds' shells, hæmatoporphyrin occurs as such. For a list of the animals in which polyperythrin was found, see Professor MOSELEY's Paper, *loc. cit.* [Since the above was

a fourth band can be seen nearer the violet which is also present in the spectrum of neutral hæmatoporphyrin. Hence polyperythrin is closely related to, if not identical with, hæmatoprophyrin, and is closely related to my actiniohæmatin, as this can also be made to yield hæmatoporphyrin ; so that all these observations fit into each other, and show, what I have endeavoured to point out, that in the lower animals we meet with pigments which resemble, or are identical with, decomposition products of hæmoglobin, and yet are built up from a body of less complex constitution than it is.*

In conclusion, I beg to thank the Government Grant Committee of the Royal Society for the grant which enabled me to purchase the instruments, reagents, and specimens required for this research.

Explanation of the Charts.

CHART I., Plate 11.

Sp. 1. Histohæmatin in the ovary of *Uraster rubens*.
Sp. 2. Modified histohæmatin in its stomach-wall.
Sp. 3. Histohæmatin in foot of *Littorina littorea*.
Sp. 4. Do. in foot of *Purpura lapillus*.
Sp. 5. Do. in mantle of *Mytilus edulis*.
Sp. 6. Do. in ovary of same.
Sp. 7. Spectrum of *receptaculum seminis* of *Helix aspersa*.
Sp. 8. Histohæmatin in the nephridium of *Limax flavus*.
Sp. 9. Do. in the nephridium of another *Helix*.
Sp. 10. Do. in the albumen gland of *Limax*.
Sp. 11. Do. in the *vas deferens* of same.
Sp. 12. Do. in stomach-wall of *Astacus fluviatilis*.
Sp. 13. Modified histohæmatin in the stomach-wall of another *Astacus*.
Sp. 14. Histohæmatin in green gland of *Astacus*.
Sp. 15. Do. in the branchiæ of some specimens of same.
Sp. 16. Do. in the green gland of *Homarus vulgaris*.
Sp. 17. Hæmochromogen-like spectrum in the testes of the beetle, *Staphylinus olens*.

written I have found hæmatoporphyrin in *Lumbricus*, and proved its identity with polyperythrin. See 'Journal of Physiology,' vol. vii., pp. 240 *et seq*.—June 16, 1886.]

* Perhaps the great molecular complexity of hæmoglobin is due more to its proteid than its coloured constituent. H. Struve tries to show that one of the *colouring matters* of hæmoglobin is a feeble acid probably combined in the blood with soda or some organic base, and proposes to name it *hæmatic acid*, another agrees with Hoppe-Seyler's hæmatin. (= hæmic acid of Struve). The blood-crystals would be, therefore, crystals of a blood-albumin coloured by these acids. Journ. prakt. Chemie, [2], xxix., 305.

CHART II., Plate 11.

Sp. 1. Histohæmatin in kidney and liver of *Tinca vulgaris.*
Sp. 2. Do. in stomach wall of *Rana temporaria.*
Sp. 3. Do. in liver of Axolotl (*Siredon pisciformis*).
Sp. 4. Do. in kidney of same.
Sp. 5. Histohæmatin in stomach-wall of same.
Sp. 6. Do. in kidney of *Trionyx.*
Sp. 7. Do. in stomach-wall of *Emys Europæa.*
Sp. 8. Do. in kidney of *Lacerta viridis.*
Sp. 9. Do. in stomach-wall of same.
Sp. 10. Do. in liver of same.
Sp. 11. Do. in kidney of pigeon (*Columba livia*).
Sp. 12. Do. in kidney of the snowy owl (*Nictea nivea*).
Sp. 13. Do. in kidney of the *Guinea-pig.*
Sp. 14. The hæmochromogen-like spectrum of the medulla of the adrenals of same animal.
Sp. 15. Histohæmatin in the intermediate zone of same adrenal.
Sp. 16. Do. in the blood-free cortex of kidney of *Rat.*
Sp. 17. Do. in the stomach-wall of same.

CHART III., Plate 12.

Sp. 1. Histohæmatin in liver of *Rabbit.*
Sp. 2. The hæmochromogen spectrum in the medulla of adrenal of same animal.
Sp. 3. Histohæmatin in the kidney of same.
Sp. 4. Do. in blood-free stomach wall of *Cat.*
Sp. 5. Do. in blood-free pancreas of same.
Sp. 6. Do. in blood-free liver of same.
Sp. 7. Do. in blood-free kidney of same.
Sp. 8. Spectrum of same animal's ovary blood-free.
Sp. 9. Enfeeblement of hæmochromogen bands in medulla of adrenals of same animal after injection with salt solution.
Sp. 10. Myohæmatin of *Musca vomitoria.*
Sp. 11. Modified myohæmatin from *Lucanus cervus.*
Sp. 12. Myohæmatin of *Creophilus maxillosus.*
Sp. 13. Do. of *Geotrupes stercorarius.*
Sp. 14. Modified myohæmatin in *Bombus terrestris.*
Sp. 15. Normal myohæmatin in same species.

Sp. 16. Myohæmatin in the spider—*Tegenaria civilis.*
Sp. 17. Do. in the heart of *Astacus fluviatilis.*
Sp. 18. Do. in the heart of *Cancer pagurus.*

CHART IV., Plate 12.

Sp. 1. Myohæmatin in the heart of *Homarus vulgaris.*
Sp. 2. Do. in the heart of *Limax flavus.*
Sp. 3. Do. in the pharyngeal muscles of same.
Sp. 4. Do. in both situations in *Arion ater.*
Sp. 5. Do. in the heart of *Helix aspersa.*
Sp. 6. Do. in the heart ventricle of a mackerel (*Scomber*).
Sp. 7. Do. in the heart of *Rana temporaria.*
Sp. 8. Do. in the heart of *Bufo vulgaris.*
Sp. 9. Do. in the heart of a *Trionyx.*
Sp. 10. Do. in the heart of *Lacerta viridis.*
Sp. 11. Do. in the heart of snowy owl (*Nictea nivea*).
Sp. 12. Do. in the heart of a *Guinea-pig.*
Sp. 13. Do. in the heart of a *Hare.*
Sp. 14. Do. in the heart of a *Dog.*
Sp. 15. Do. in the heart of a *Rat.*
Sp. 16. Do. in a *musculus papillaris* of *human* heart.
Sp. 17. Isolated myohæmatin in muscle plasma.
Sp. 18. Changed myohæmatin isolated by pepsine, in water, treated with NH_4HS.

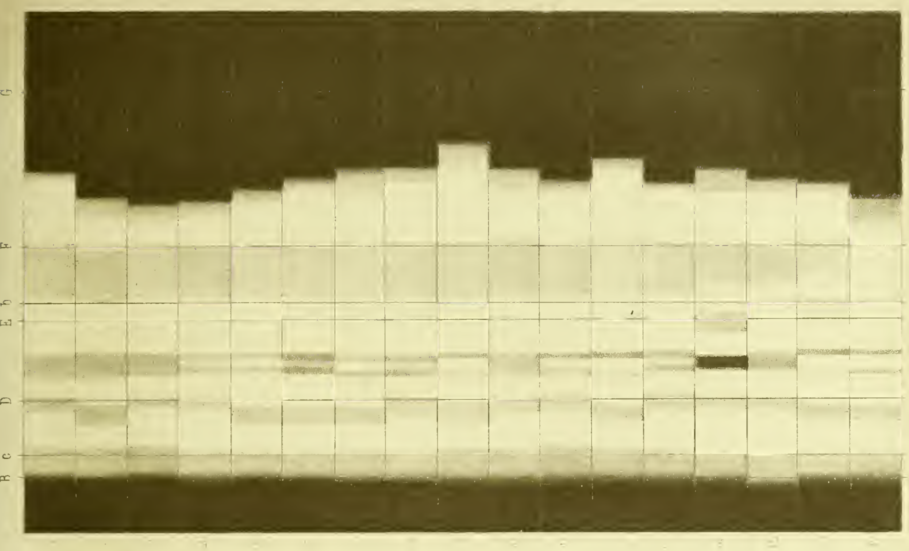

Chart II

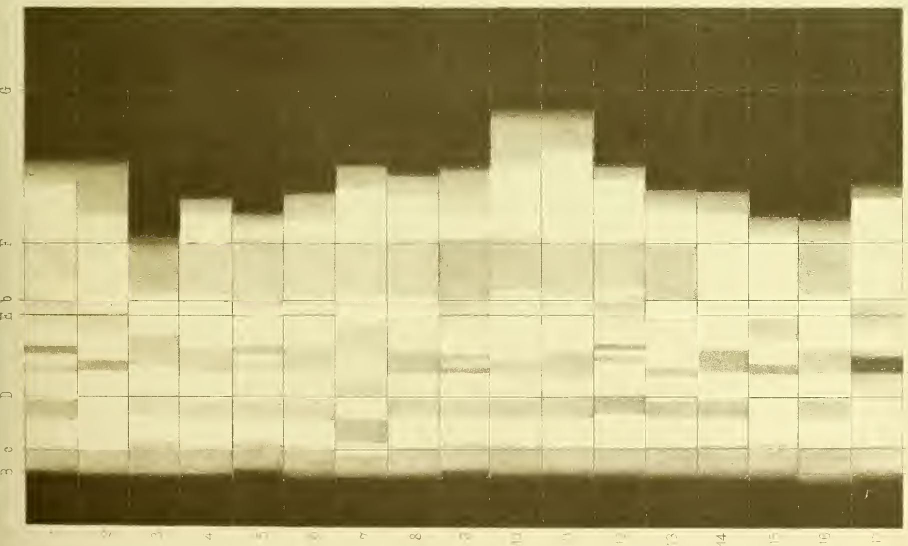

Chart I

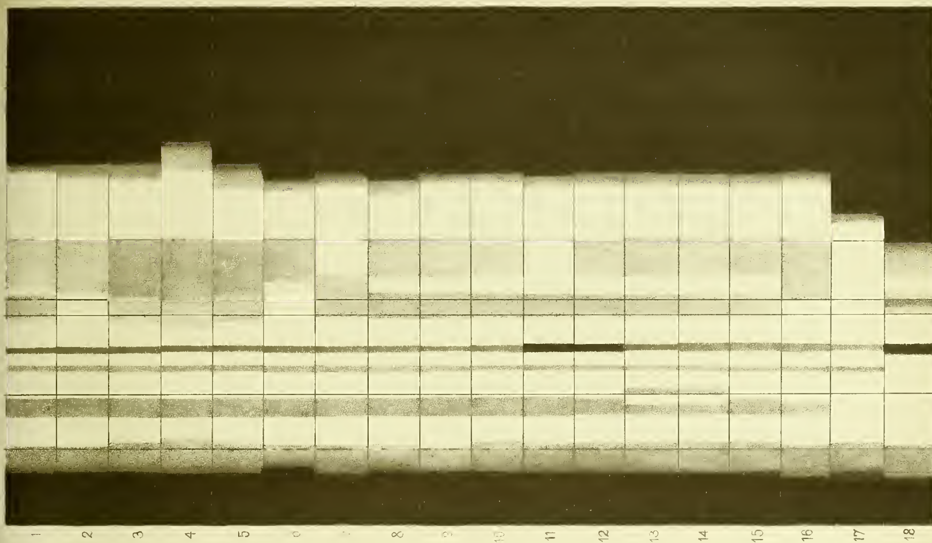

Chart IV

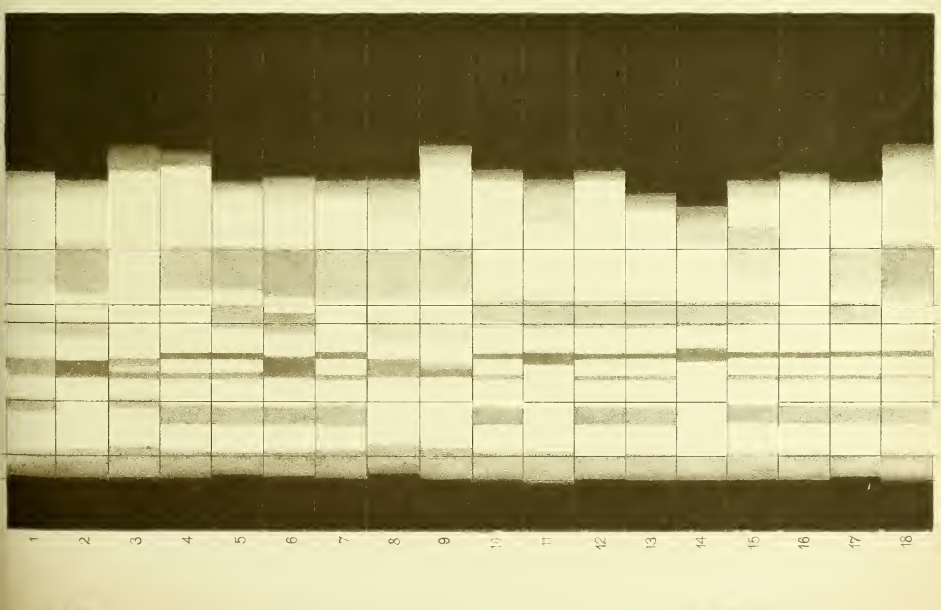

Chart III.

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